The Dutch Experience with Reduction of Bacterial Contamination of Platelet Products

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OVERVIEW

- Introduction
- Pre-storage pooling: the buffy-coat principle
- Screening results 2002-2003
  - Standard procedure; diversion results; changed disinfection
- Prolonged storage time
- Validation aspects
- Implementation lessons
- Recommendations

BACKGROUND

- Awareness on bacterial contamination of blood products increased (not only Europe)
  - 15-30 % of deaths related to transfusion caused by bacterial contamination (Transfusion Transmitted Bacterial Infection; TTBI)
- Risk for TTBI much higher than for viral transmission
- Platelet concentrates recognized as main risk

Screening in Europe

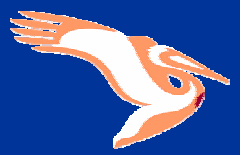
- Sweden/Danmark/Norway: 60 – 100 %
- Belgium: since 1998 100 % mandatory
- Netherlands: since November 2001(some centers started before) mandatory
- Other countries: 1-2 % QC, some individual centers higher rate. In several countries under discussion, but sofar no obligations
- Focus on the Netherlands

The Netherlands

- Inventarisation of actual risk for bacterial contamination of blood products resulted in:
- Advice to introduce bacterial screening for thrombocyte concentrates
  - Culture for 7 days (using BacT/Alert)
  - Release as “negative to date”
  - indirectly: increase of QC for related products
  - collect data for haemovigilance
- Advice accepted by Health Authorities and screening implemented per November 2001 (the perfect etc.)

Some facts

- Netherlands 93 % buffy coat derived PC
- Apheresis mainly for HLA-typed donations
- 100 % screening: release as ‘negative to date’
- BacT/Alert; aerobic and anaerobic bottle, inoculated with 7.5 ml each
- Sampling for BC-PC: within 2 h after preparation, but this is 18-24 h after whole blood collection
- Sampling for Apheresis PC: within 12 h



Preparation of Platelet Concentrates

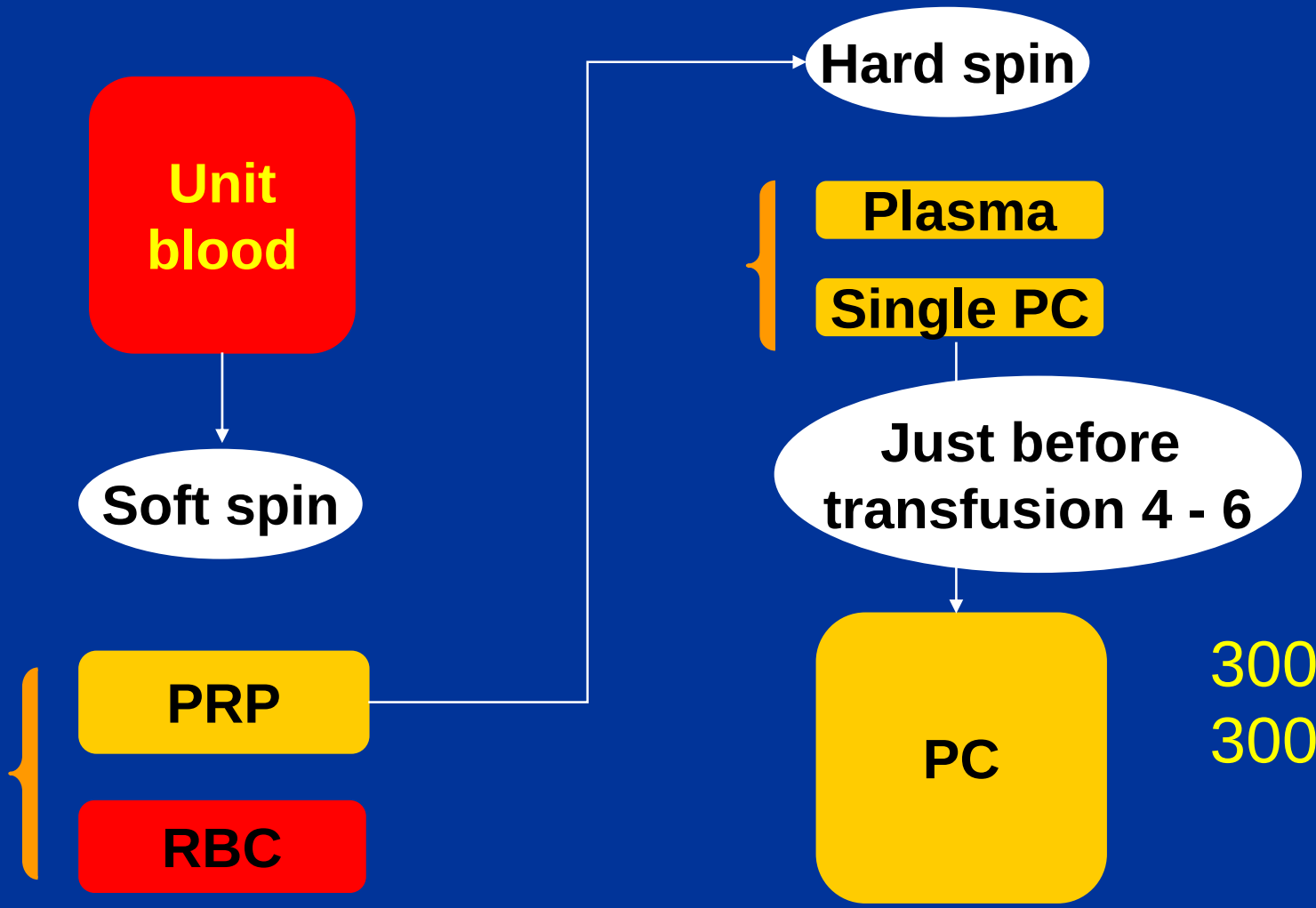
Whole blood derived:

- **PRP method: Mainly North-America; single, but movement towards pre-storage pooling**

2. **BC method: Mainly Europe; single (1980 – 98) and pooled (1995 – now). Recently Canada**

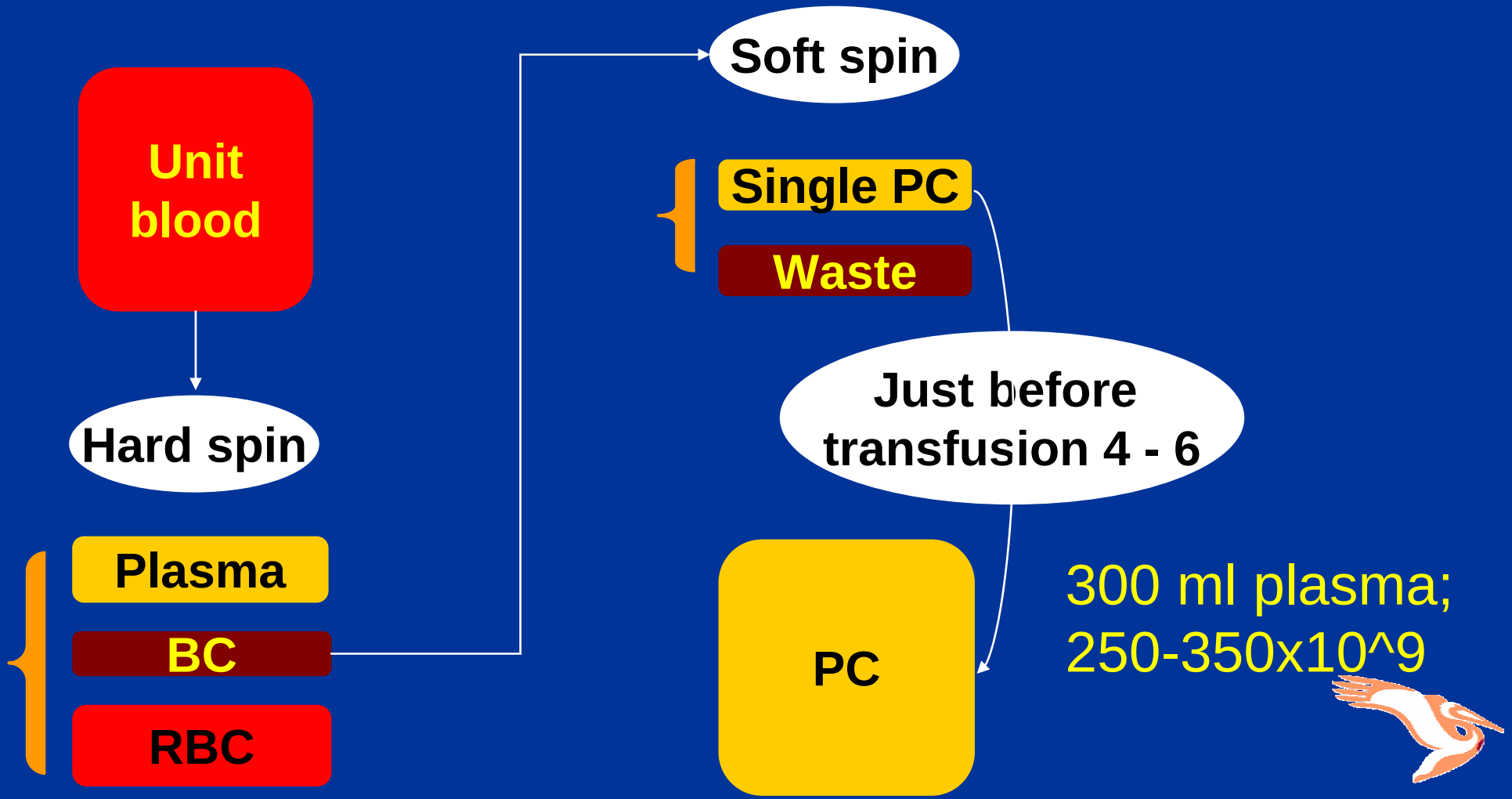
Platelet apheresis

PRP method

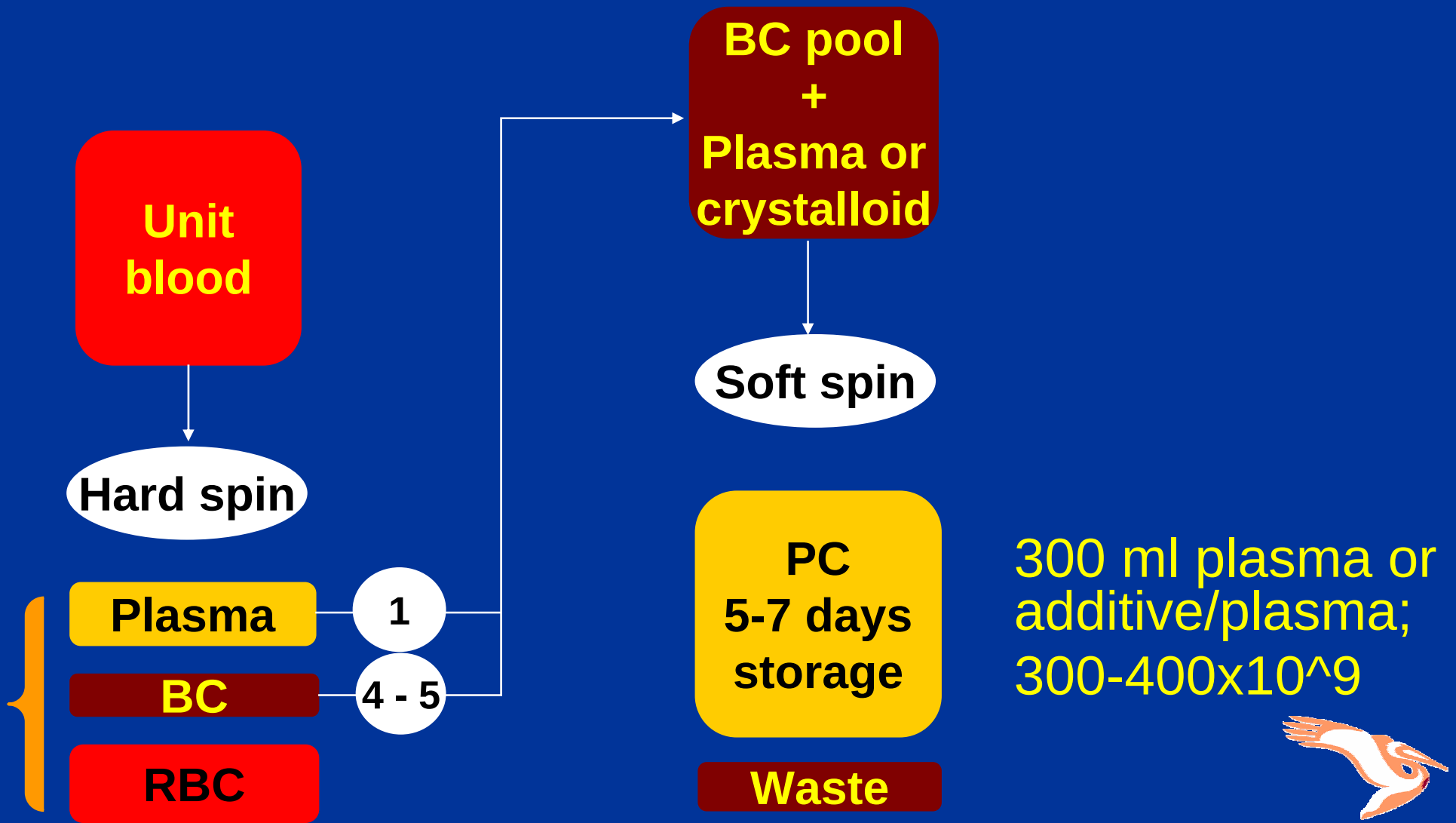


300 ml plasma;
300-400x10^9

Single BC method



Pooled BC method



300 ml plasma or additive/plasma;
300-400x10^9

Differences in PRP and BC PC

	PRP-PC	*Single BC-PC*	*Pool BC-PC*
WBC.	**5-25%WB**	**<0.5% WB**	**<0.5% WB**
Plt. Activation.	**+**	**-**	**-**
Plt. yield	**60-75%**	**50-65%**	**60-75%**
Plasma yield		**+ 75 ml**	**+75 ml or +375 ml**
Pooling	**Post-storage**	**Post-storage**	**Pre-storage**

Pooled BC PC

1. Pooling during preparation = pre-storage

2. Acceptable in vitro characteristics during storage for up to 7 days (Vox Sang. 1994 34 311- 316)

3. No effect on availability due to pooling, production faster and easier than single BC PC

4. No delay due to bacterial testing, because of ‘negative to date’ principle and sampling < 2 h after preparation

Additional remark : Screening has some effect on availability of apheresis PC, often directed (HLA-matched) donations

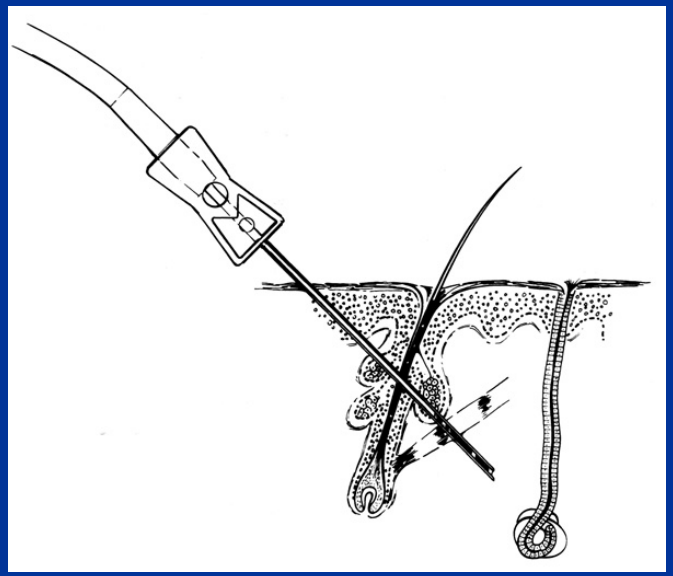
Dutch Results of Screening

Diversion

- **One center, on experimental basis, introduced diversion pouch for whole blood collections**
- **All centers used diversion pouch for apheresis collections**

DIVERSION OF FIRST FLOW

- Study of effect upon bacterial contamination of whole blood units after diversion of first 10 ml.
- Idea: contamination is mainly with common skin flora, ‘skinplug’ is important cause.



Reproduced from Blajchman, Vox Sang.74S2,1998

RESULTS OF WB DIVERSION STUDY

- In total 7000 units of whole blood tested.
- Whole blood unit sampled for BacT/Alert after diversion of first 9.6 ml.
- Degree of contamination: 0.21 %

  95 % confidence interval: 0.12 - 0.35 %
- Base level 0.36 % (18,000 units; 0.25 - 0.44)
- Significant decrease (p = 0,046)

De Korte et al. Vox Sang. 2002, July

RESULTS OF DIVERSION STUDY

- Open question: has diversion during blood collection also an effect on the contamination of the end product, the Platelet Concentrate from a pool of 5 buffy coats
- Need for a special collection configuration; used in a study from Blood Center Gelderse Rivieren (Region South East).

diversion of first 25-30 ml

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diversed volume to be used for test purposes

Dutch Results of Screening

Method of disinfection

- **During last Quarter of 2002: implementation of double swab disinfection method with isopropyl alcohol**
  - **Various papers indicated double swab with 30 sec spacing was more effective than single application**
  - **Arguments contra iodide won**
  - **Before change: most centers single swab**

Dutch Results of Screening

2.5 year experience (some centers up to 6 years) with 100 % screening of platelet concentrates

- Results before vs. after change in disinfection
- Results with diversion; effect of disinfection change
- Results for Apheresis units; effect of disinfection change
- Overall comparisons

	Standard collection of whole blood Period Jan 2002 – Oct 2003	
	Various disinfection	Standardized double swab*
Total BC PC tested	42583	46544
Initially positive (%)	407 (0.96)	381 (0.82)
Confirmed positive (%)	381 (0.89)	347 (0.75)
No subculture from positive bottle	26 (6.3 % of flagged positives)	34 (8.9 % of flagged positives)

*30 sec spaced 70% isopropylalcohol swabs

Before mainly 0.5% chloorhexidine/ 70% ethanol swab(s)

	Collection of whole blood with diversion pouch Period Jan 2002 – Oct 2003	
	Old disinfection	Standardized double swab*
Total BC PC tested	4362	4446
Initially positive (%)	22 (0.50)	16 (0.36)
Confirmed positive (%)	18 (0.41)	11 (0.25)
No subculture from positive bottle	4 (18 % of flagged positives)	5 (31 % of flagged positives)

*30 sec spaced 70% isopropylalcohol swabs

	Apheresis PC with diversion pouch Period Jan 2002 – Oct 2003	
	Various disinfection	Standardized double swab*
Total apheresis PC tested	3037	3742
Initially positive (%)	7 (0.23)	12 (0.32)
Confirmed positive (%)	6 (0.20)	10 (0.27)
No subculture from positive bottle	1 (14 % of flagged positives)	2 (17 % of flagged positives)

*30 sec spaced 70% isopropylalcohol swabs

Comparisons

- Effect of diversion: highly significant for BC PC
  - old disinfection: 0.96 vs 0.50 (p=0.004)
  - new disinfection: 0.82 vs 0.36 (p=0.001)
- Double swab disinfection: 0.96 vs 0.82 (p=0.03) for standard collection; not significant for diversion or apheresis (lower numbers)
- Apheresis vs pooled BC-PC not longer different with both diversion and double swab: 0.32 vs 0.36 %

Subcultures from positively flagged

- Differentiation results after diversion are different, as described for whole blood diversion study (de Korte et al.)
  - Relatively less CNS and more Propioni sp
  - Increase of percentage with failure to grow in subculture
  - Similar trend for changed disinfection
  - Percentage of ‘dangerous’ bugs (rapid growers) decreased more than overall percentage

Negative to date vs. quarantaine

- In practice similar results:
- Quarantaine for 2 days would have prevented 90 % of PC with fast-growing bacteria to be released
- For > 90 % of cultures with fast-growing bacteria product was still in Blood Center upon positive signal
- Products with positive signal after release mainly slow-growing, like Propioni sp and diphteroid rods

Related Erythrocyte Concentrates

- About 70 % is still in Blood Center stock
- Recall in 75 % of cases succesful (in hospital blood bank)
- Overall 92 % of related EC prevented from being transfused (minimum value)
- Positive culture in related erythrocyte concentrate in 45 % of cases with a positive culture for PC; same microorganism

Related Erythrocyte Concentrates

Species	# cases*	RCC pos	RCC neg
CNS	143	20	123
Bacillus	24	3	21
Diphteroid rods	47	25	22
Propioni sp	134	110	24

* Number of cases in which RCC were cultured when a PC was found to be contaminated with the specified organism

Releated RCC

- Theoretical 20 % of RCC contaminated (5 RCC per BC PC)
- In practice less than 10%
- Mainly Propioni sp survive in RCC, CNS has much lower probability to survive and to result in positive culture

Sanquin Policy Changes

- Starting June 2004 all collections should be performed with system including diversion pouch
- Since January 2004 BC PC in plasma have a shelf-life of 7 days

Prolonged Storage of PC

- For prolonged storage: main concern is bacterial contamination; minimized by screening
- If validated with respect to in vitro quality of platelets: prolonged storage in combination with culture was allowed in the Netherlands (Sweden, Norway), but:
  - Should be supported by *in vivo* data
  - not all physicians believe that 7 days old platelets are as effective as fresh platelets: need to prove efficacy

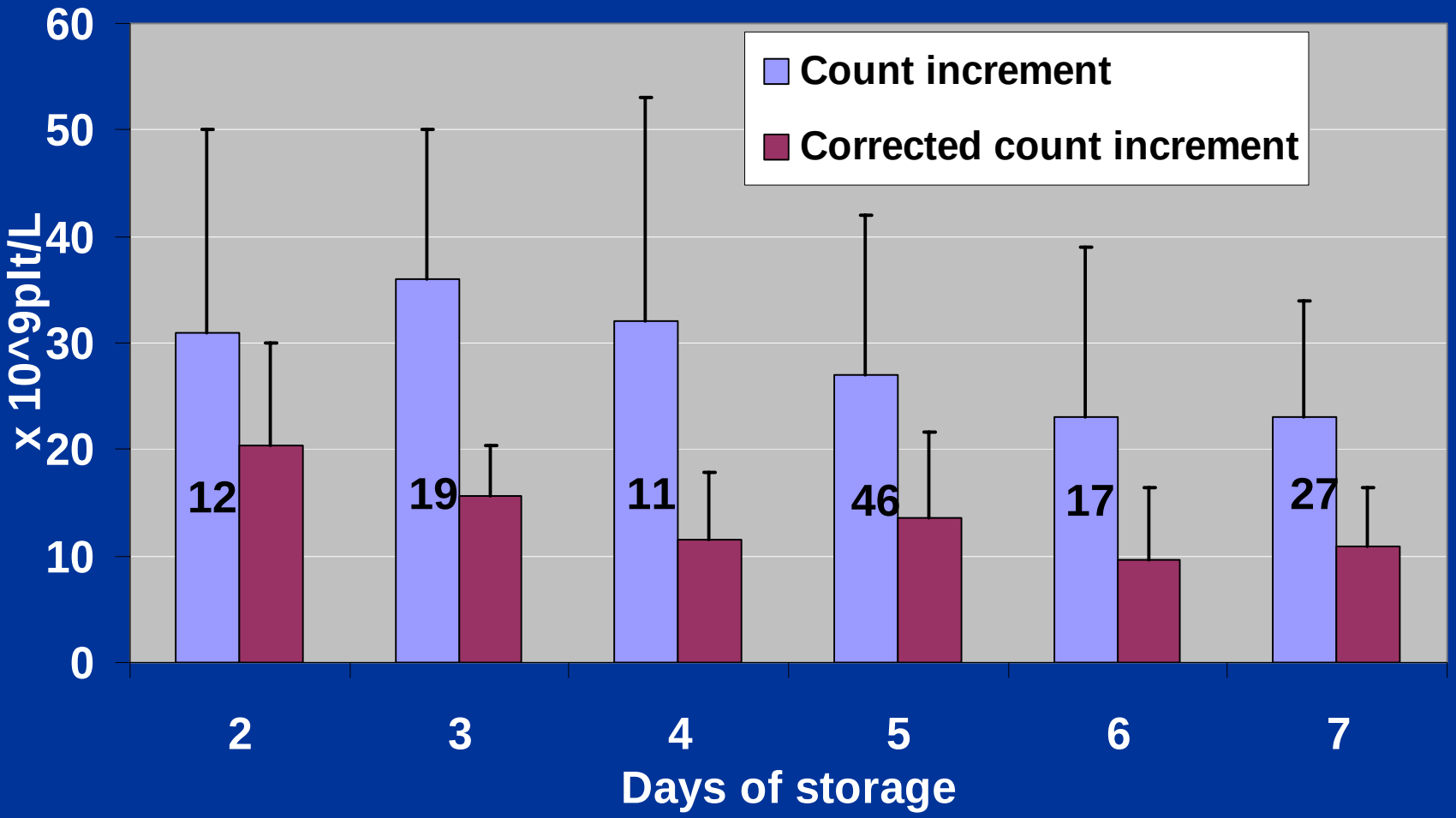
Prolonged Storage of PC
In Vitro quality

- Multiple studies showing that under various conditions day 7/8 is maximally 20 % worse compared to day 5 (providing use of right containers)
- 7 days is also possible with use of additive solutions and variable amounts of plasma cross-over (10-40 %)
- *in vitro* only improved compared to 1986 (7 to 5 days)
- Also pre-storage pooled PRP has very acceptable in vitro quality after 7 days (Vox Sang. 1995 68 82-89)

Prolonged Storage of PC clinical study

- Blood Bank North West + Free University Academical Hospital performed a clinical study with determination of Count Increments (1 hour after transfusion).
- hemato-oncological patients; no serious bleedings
- PC in plasma from 5 pooled BC
- storage during 2–7 days (variable number of transfusions)
- Recently published in Transfusion 2004 44 330-336
- Based on this publication 7 days is now authorized in the Netherlands (with post-transfusion surveillance)

Prolonged Storage of PC



data from Transfusion 2004 44 330-336

Prolonged Storage of PC

- Both *in vitro* and *in vivo* data support that PC **(BC derived, in plasma)** stored for 7 days still have a good quality and can be used for patient care to overcome logistical problems; Official authorization in the Netherlands

- Extension of shelf life from 5 to 7 days; out-dating will greatly reduce; first experience at least 10 % reduction (with 5 days 15 – 25 %, with 7 days 5 – 10 %)
- Financial benefit, screening pays itself

Validation aspects

- Selectivity or False positives
- Sensitivity or False negatives

Sample drawing / inoculation

- Sampling using integrated sampling pouch with needle or adapter to fit culture bottle adapter

Different types of “False positives”

- **Accidental Contamination by processing:**
- **Aseptic procedure results in low number accidentally contaminated bottles: 0 out of 2000 procedures: < 0.05%**
- **Negative confirmation culture**
- **36 out of 474 positively flagged cultures; bug not growing under standard culture conditions or system failure**
- **Temporarily positives**
- **Upon reculture of PC flagged positive; 20 - 50 % again positive (limited number studied); ‘self-sterilization’**

False negatives

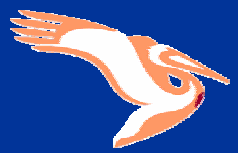
- **Bug not recognized by culture system**
  - Extensive studies in literature (for example Brecher et al.) indicate that all bugs thought to be relevant are picked up
- **System not sensitive enough**
  - Extensive studies showed that sensitivity is 1-10 CFU/bottle, with 7.5 ml inoculated: 0.2 - 1 CFU/ml of PC will give positive signal
  - For < 4 % of positive PC both bottles are positive, indicating that you are on lower limit of sensitivity
  - Too early sampling: from QC data in outdated products frequency is much lower (indicating false positives rather than false negatives)

Validation aspects
Conclusions

- **Selectivity**
- **Relatively low, but not in classical meaning**
  - **Bugs are not always surviving in actual products**
  - **Fraction of positives would have caused clinical problems**
  - **Product changes during testing; not simple repeat**
- **Sensitivity**
- **Very high sensitivity with chosen approach, but can we afford to go lower?**

Implementation lessons

- Motivation of all involved people
- Good relations with clinic
  - Acceptance in clinic of ‘negative to date’
  - Acceptance in clinic of ‘positive but already transfused’
  - Acceptance in clinic of ‘related RCC might be positive, but with low possibility’
- Training of involved personnel in microbiology
- Standardization
  - Sampling and inoculation method
  - Inoculation time



Recommendations

- For all platelet products: use sensitive detection method with ‘negative to date’ release
- For whole blood derived platelets: Change to buffy-coat PC or at least introduce pre-storage pooling of PRP PC
- In case of transfused product with clinical symptoms: use fact that blood center is ahead, help with determination of possible resistance
- Haemovigilance: monitor the effects

Final Conclusion

- Based on experience sofar: implementation of a system for bacterial screening is found to be very succesful
  - Easy monitoring of possible improvements
  - Allowing shelf-life prolongation
  - Reduction of clinical cases
  - Quick adaptation in clinic
- By the combination of diversion and improved desinfection BC PC became similar to apheresis PC with respect to bacterial contamination degree as detected in screening; important argument for whole blood derived platelets